Chapter

Terpenoids as Important Bioactive Constituents of Essential Oils

Fongang Fotsing Yannick Stephane
and Bankeu Kezetas Jean Jules

Abstract

Plant and plant-derived natural products have a long and significant history in traditional medicine all over the world. Many studies in the recent past years focused on the benefic properties of essential oils (EOs) and their major components, terpenes and terpenoids (that are mostly monoterpenes and sesquiterpenes), and their biological properties. This chapter focuses on terpenoids as important bioactive constituents of EOs. It describes their uses, importance, extraction processes, and classification. The chapter provides an in-depth overview of the latest findings/research about terpenoids in EOs. It contains a well-prepared background, introduction, classification, chemical tests, bioactivities, as well as the characterization of terpenoids. It also discusses the bioactivities of EOs and that of terpenoids, with regard to their synergetic and/or their antagonistic effects.

Keywords: monoterpenoids, sesquiterpenoids, diterpenoids, terpenes, terpenoids, essential oils, bioactivities

1. Introduction

The use of plant and plant-derived natural products for medicinal, religious, and cosmetic purposes has a history dating back to the emergence of humanity. Exploring natural plant products as an option to find new chemical entities as leads is one of the fastest growing areas of research. Medicinal plants are rich sources of bioactive phytochemicals and/or bionutrients, which have shown important role in preventing chronic diseases like cancers, diabetes, and coronary heart diseases [1]. It is well documented that plants produce these chemicals to protect themselves, but they also protect plants from diseases and damages and contribute to the plant's color, aroma, and flavor [2]. The pharmaceutical properties of aromatic plants are partially attributed to essential oils (EOs), which can also be seen as an important group of plant secondary metabolites. Although the use of EOs has been primarily related to food flavorings, cosmetics, and perfumes due to their aroma, research demonstrates the high potential of the use of volatile monoterpene constituents to cure and prevent human diseases [3, 4]. During the recent years, plant EOs have come more into the focus of phytomedicine and aromatherapy; hence their widespread use has raised more interest to scientists in basic research, especially their antimicrobial, antioxidant, and anticancer activities. In general, EOs consist of chemical mixtures involving from several tens to hundreds of different types of molecules, most of them being complex natural mixture of terpene and

phenylpropanoids (benzene derivatives) which are responsible for their biological activities [5, 6]. At the first glance, terpenes and EOs can seem alike; both can come from plants and are aromatic; for many they are used for the same purpose. These similarities have led to a wide misconception that they are same, but this is not necessary the case [7].

2. Essential oils

2.1 Definition

A plethora of practical definitions of the term essential or volatile oils exist. Essential oils are concentrated aromatic hydrophobic oily volatile liquids characterized by a strong odor and produced by different plant materials such as flowers, peels, rhizomes, buds, seeds, leaves, twigs, bark, herbs or grass, wood, fruits, roots, and whole plant from one single botanic species [7–9]. However, EOs with a specific characteristic (including chemical properties and biological activities) are generally obtained from a single botanical source when the age of the plant, the climate, and the edaphic and harvest period are relatively identical [10]. They are called "essential oils" because they contain the "essence" of the plant material. A few are produced by animals and microorganisms [11]. Mosses, liverworts, seaweeds, and fungi have also been shown to contain EOs. EOs are limpid, rarely colored, and soluble in nonpolar or weakly polar organic solvents and of lower density (lighter) than water, with very few exceptions [12]. They are usually colorless particularly when fresh, but few may also be pale yellow (yellow mandarin), blue (*Matricaria chamomilla* well known as chamomile), orange (sweet orange, *Citrus sinensis*), and green (bergamot, *Citrus bergamia*) [13]. Nevertheless, they may be readily oxidizable with age by light, heat, or air, which resulting to the dark color [14]. Therefore, they need to be stored in a cool and dry place, preferably in amber glass containers. The primary difference between terpenes and EOs is that they contain terpenes and a variety of other compounds as well.

2.2 Distribution, uses, and importance

The quality and the quantity of EOs in plant material depends on the climate, the soil type, the age and vegetable cycle stage, the preparation method, chemotypes, as well as the plant organ [8]. An estimated 3000 EOs, from about 2000 plants, are of great value and are used in a very large variety of fields [15, 16]. All plants possess principally the ability to produce volatile compounds, quite often, however, only in traces. Those plants that can produce an EO of commercial interest are called essential oils plants [17]. EOs occur specially in higher plants (with about 17,500 known species) but are distributed in good amount in a limited number of families including Myrtaceae, Myristicaceae, Oleaceae, Rosaceae, Acoraceae, Cupressaceae, Lauraceae, Compositae, Rutaceae, Lamiaceae, Asteraceae, Umbelliferae, Apiaceae, Poaceae, Zingiberaceae, etc. [18–21].

In most cases, the biological function of EOs remains obscure. They are nowadays subject of intensive scientific research and also attract attention of diverse industries due to their potentials as active pharmacological compounds or natural preservatives [22]. Their ecological role is however well studied and described. The most known are plant interactions (allelopathic agents, germination inhibitors) and plant–animal interactions for protection against predators (insects, fungi, herbivores) and attraction of pollinating insect to their host [23]. Industries have always

had special interest on the microbial safety of cosmetics, as microbial spoilage can lead to product degradation and cause a risk for customers' health. EOs and drugs containing them are of great importance in pharmacy, perfumery (heal, perfume, incense, household cleaning products), food technology (favor for food, drinks, spices, preservative), agriculture (insecticide), and aromatherapy. Their importance is nowadays known and appreciated in plant chemotaxonomy [24, 25].

2.3 Extraction and analysis

2.3.1 Extraction of essential oils

The world production and consumption of EOs and perfumes are increasing very fast. Production technology of EOs is an essential element to improve their overall yield. They are obtained from raw material by several extraction techniques such as water or steam distillation, solvent extraction, expression under pressure, microwave-assisted extraction, supercritical fluid, or subcritical water extractions [22, 26–28]. The best extraction method to use depends on the ease of evaporating (volatility) and the hydrophilicity or hydrophobicity (polarity) of the desired components. The extraction method chosen greatly affects the chemical composition of EOs.

2.3.1.1 Classical and conventional methods

They are the most frequently used method for the extraction of EOs from plants.

2.3.1.1.1 Hydrodistillation

It is the oldest and easiest conventional method of extraction of EOs [11, 29–31]. The principle is based on the isotropic distillation. The plant material soaks up water during the boiling process, and the oil contained in the oil cells diffuses through the cell walls by means of osmosis. The distillation time depends on the plants material being processed (**Figure 1**).

2.3.1.1.2 Steam distillation

The principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100°C so that the volatile components with the

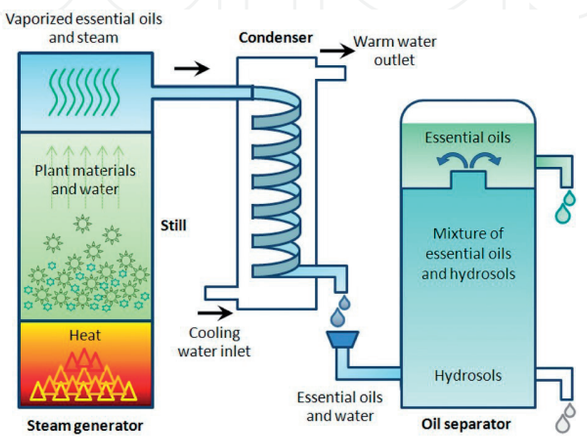

Figure 1.
Diagrammatic illustration of hydrodistillation (HD) method [32].

boiling points ranging from 150 to 300°C can be evaporated at a temperature close to that of water. The steam distillation takes advantage of the volatility of a compound to evaporate when heated with steam and the hydrophobicity of the compound to separate into an oil phase during the condensation process (**Figure 2**) [33].

2.3.1.1.3 Solvent extraction

Also known as liquid–liquid partitioning, its principle is based on the solubility in an organic solvent non-mixable to water. This technique is used on delicate plants to produce higher amounts of EOs at a lower cost. The method is limited by the compound solubility in the specific solvent used, long extraction time, relatively high solvent consumption and often unsatisfactory reproducibility and purity (**Figure 3**) [33].

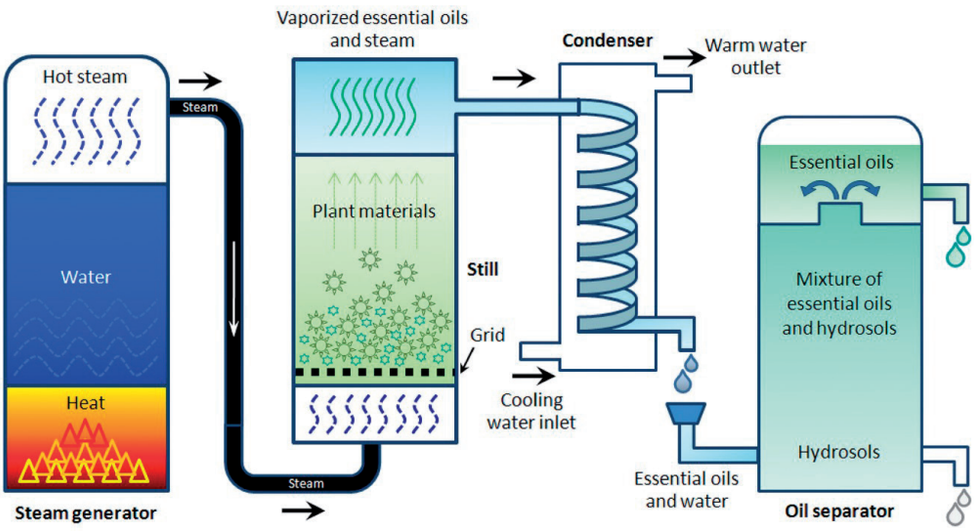

Figure 2.
Diagrammatic illustration of steam distillation method [32].

Figure 3.
Illustration of liquid–liquid extraction method.

2.3.1.1.4 Soxhlet extraction

Typically, it is a solid–liquid extraction used when the desired compound has a limited solubility in a solvent and the impurity is insoluble in that solvent. There are several advantages of using this technique. These advantages include:

- Low solvent consumption for a larger amount of raw material,

- Repeatedly brought into contact with fresh portions of the solvent, this prevents the possibility of the solvent to become saturated with extractable material and enhances the removal of analyte from the matrix. Moreover, the temperature of the system is close to the boiling point of the solvent. This helps to increase the extraction kinetic of the system.

As disadvantages, it requires several hours or days to be performed; moreover, the sample is diluted in a large volume of solvent.

Due to heating, the thermal degradation and volatilization of components have been observed, and hydrolysis of esters to yield alcohols and carboxylic acids can occur (**Figure 4**) [34].

2.3.1.1.5 Cool pressing method

Also kwon as scarification method, this is one of the best methods to extract EOs. The term cool pressed theoretically means that the oil is expeller-pressed at low temperature and pressure. This process insures that the resulting oil is 100% pure and retains all the properties of the plant. Here the heat is reduced and minimized throughout the batching of the raw material. EOs are then separated from the material by centrifugation [36].

Since economy, competitiveness, eco-friendly, sustainability, operation costs, high efficiency, and good quality become keywords of the modern industrial production, the development of EO extraction techniques has never been interrupted. The most relevant disadvantage of conventional techniques are time and solvent consumption and also related to the thermolability of EOs components which undergo chemical alteration (hydrolyze, isomerization, oxidation) due to the high

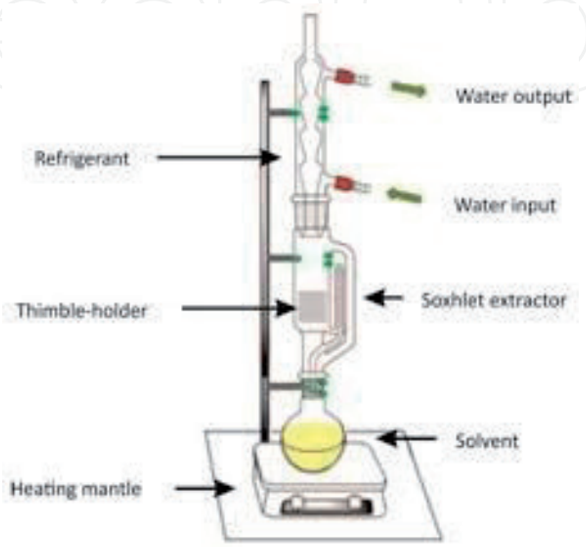

Figure 4.
Soxhlet equipment [35].

applied temperatures [37]. The quality of the obtained oil is damaged, particularly if the extraction time is long. It is important that the extraction method maintain the chemical composition and the natural proportion at its original state. Strictly speaking, conventional methods are not the only way for the removal of EOs. Novel techniques known as innovative have been developed for this purpose but may not necessarily be widely used for commercial production due to the high cost of production of oils without any alteration of their thermosensitive components (**Figure 5**).

2.3.1.2 Innovative techniques of extraction (nonconventional)

2.3.1.2.1 Supercritical fluid extraction

It is a process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. In practice, more than 90% of all analytical supercritical fluid extraction (SFE) is performed with carbon dioxide (CO_2) as the most used fluid. The CO_2 is chosen for several reasons including the following: relatively low critical pressure (74 bars) and temperature (32°C), inertness, non-toxic, nonflammable, high soluble, non-corrosive, safe, available in high purity at relatively low cost, perfect conditions for thermosensitive compounds extraction, selectivity for desired compounds, and easy removal from the extract. At lower temperatures, to avoid potential damage of desired components of EOs, supercritical CO_2 extraction technique is highly recommended [39, 40]. Extraction of EOs by SFs, particularly with CO_2, provides products free of toxic waste, having a higher quality (especially it reserves the thermal instability of compounds) than EOs obtained by conventional methods (**Figure 6**) [40–42].

2.3.1.2.2 SFE assisted by cold pressing (SFEAP)

SFEAP is a novel technique of extraction recently developed by Johner and collaborators [43]. It integrated both the cold-pressed extraction method and the SFE technique. Here, the solid raw material is loaded inside the extraction vessel, and

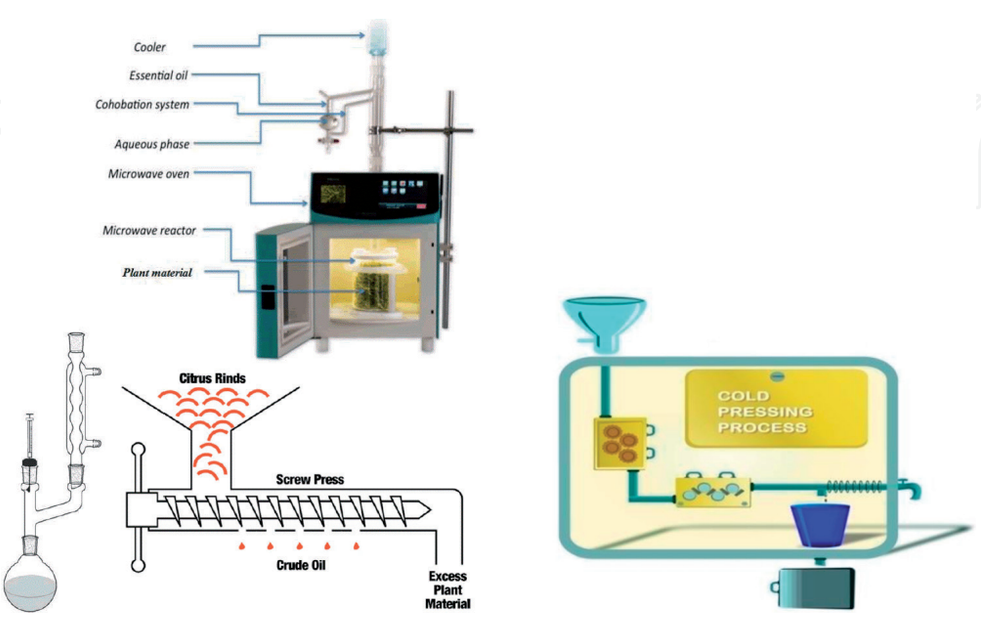

Figure 5.
Cold pressing apparatus and procedure distillation method [34, 38].

a cold pressing is provided by contracting an under pressure piston with the raw material. SFEAP has been shown to offer faster extraction rate at 333 K and 40 MPa with the best yield [44]. Its advantages include gain of extraction time and solvent consumption. This technique has been used to extract EOs from *Foeniculum vulgare*, *Caryocar brasiliense*, and clove (**Figure 7**) [43–45].

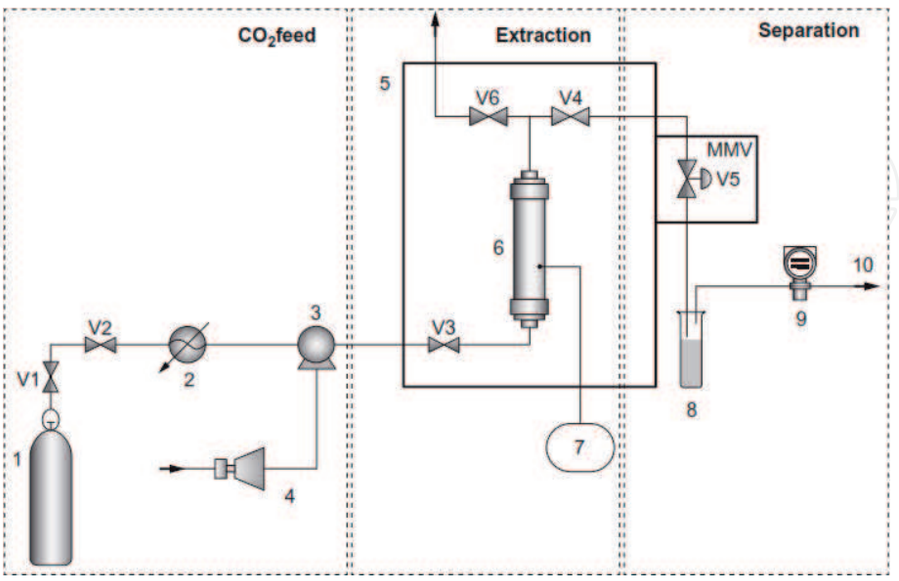

Figure 6.
Flow diagram of SC-CO_2 extraction [40]. (1) CO_2 cylinder, (2) cooling bath, (3) pump, (4) compressor, (5) oven, (6) extractor vessel, (7) monitor, (8) collecting bottle, (9) flowmeter, and (10) CO_2 outlet. V1–V_6 flow control valves.

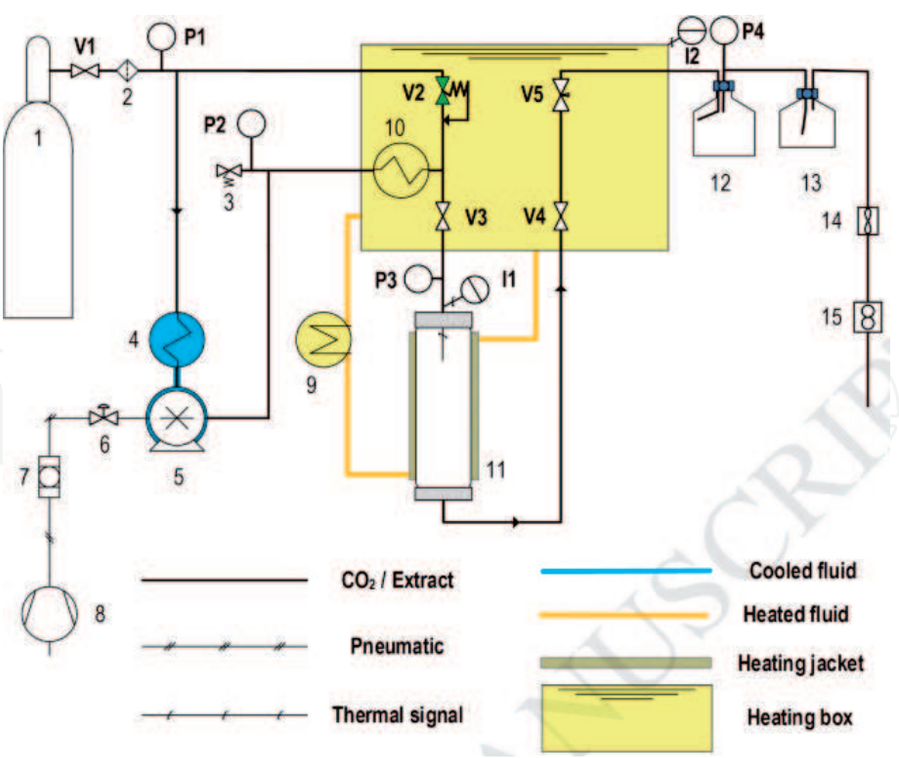

Figure 7.
Schematic diagram of SFEAP apparatus [43]. (1) CO_2 reservoir; (2) CO_2 filter; (3) safety valve; (4) cooling bath; (5) air-driven CO_2 pump; (6) control (air flow); (7) air filter; (8) air compressor; (9) heating bath; (10) serpentine tube; (11) extraction cell; (12) 1° extract collecting vessel; (13) 2° extract collecting vessel; (14) flowmeter (15) flow totalizer, V2 back pressure; V5 micrometering valve; V(1,3,4) blocking valve; P(1,2,3,4) pressure gauge; I1 temperature indicator; I2 temperature indicator.

2.3.1.2.3 Microwave-assisted hydrodistillation

The principle of the microwave-assisted hydrodistillation (MAHD) is based upon its direct impact with polar materials/solvents and is governed by two phenomena: ionic conduction and dipole rotation, which in most cases occurs simultaneously [46]. MAHD has been shown to reduce both extraction time and volume of solvent required, minimizing environmental impact by emitting less CO_2 in atmosphere [47–49]. Some recently reported studies have successfully utilized a microwave oven for the extraction of volatile active components from plants [50]. It has been regarded as an important alternative in conventional extraction techniques because of its advantages which mainly are a reduction of extraction time, solvents, selectivity, volumetric heating, and controllable heating process (**Figure 8**) [51].

2.3.1.2.4 Ultrasound-assisted extraction

The basic principle of ultrasound-assisted extraction (UAE) to extract EOs from plant raw material consist of generating sound waves (ultrasound frequency about 20 KHz), which create cavitation bubbles in the solution and produce enough energy to break the structure containing the oil in order to release it. Moreover, UAE can act as an emulsifier dispersing lipophilic molecules in water, this facilitating the subsequent separation and purification of EOs [54, 55]. This technique was developed in 1950 [56]. It has been used to extract many EOs especially from flowers, leaves, or seeds [32, 55]. As known disadvantages, it requires filtration steps, and possible degradation of compounds at high frequencies occurs (**Figure 9**) [57].

2.3.1.2.5 The microwave-assisted extraction

Microwave-assisted extraction (MAE) is a process of using microwave energy to heat the solvent in contact with a sample in order to partition analytes from the sample into the solvent. The ability to rapidly heat the sample solvent mixture is inherent to MAE and is the main advantage of this technique [59]. It is a recent green technology broadly used to extract various EOs from plant. It has been established as an alternative method to conventional heating because it allows gain of time, volume of solvent used, and amount of biomass needed while increasing

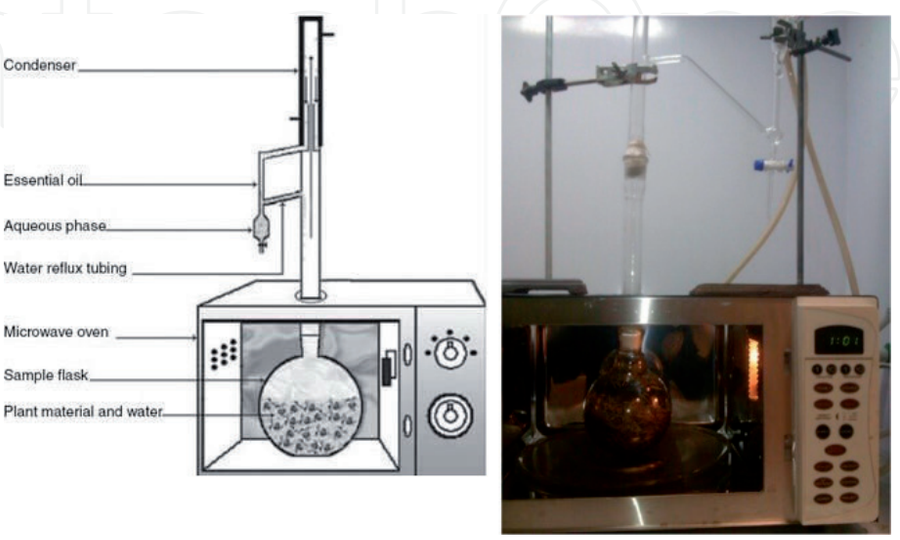

Figure 8.
Schematic and picture of MAHD apparatus [52, 53].

the extraction yield [28]. In most cases, recoveries of analytes and reproducibility are improved compared to conventional techniques (**Figure 10**) [59].

2.3.1.2.6 Solvent-free microwave extraction

Solvent-free microwave extraction (SFME) is proposed as a method for "green" extraction of edible EOs from fresh plant material, at atmospheric pressure without addition of water or organic solvent [61]. The SFME apparatus (**Figure 3**) is an original combination of microwave heating and dry distillation at atmospheric pressure. Based on a relatively simple principle, this method involves placing the plant material in a microwave reactor, without adding any solvent or water. The internal heating of the in situ water within the fresh plant material distends the plant cells and leads to the rupture of the glands and oleiferous receptacles. This process thus free EO which is evaporated by in situ water of the plant material. A cooling system outside the microwave oven condensed the distillate continuously. The excess of water is refluxed to the extraction vessel in order to restore in situ water to the plant material. At the end, EO is removed from the aqueous extract by simple decantation. SFME is neither a modified microwave-assisted extraction (MAE) which uses organic solvents nor a modified hydrodistillation process which uses a large amount of water; it can be consider as a dry distillation process, with water coming from the fresh plant material [62–64]. As advantages, the SFME

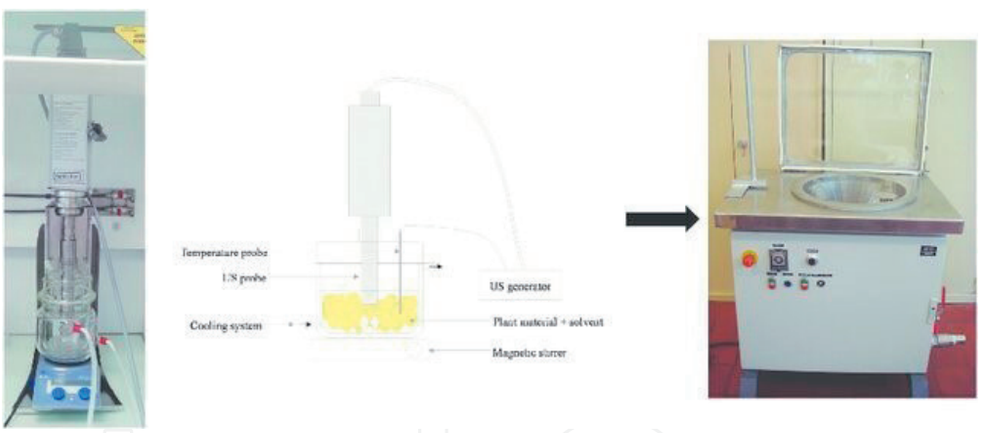

Figure 9.
Ultrasound-assisted extraction (UAE): from laboratory (a) to pilot scale (b) [58].

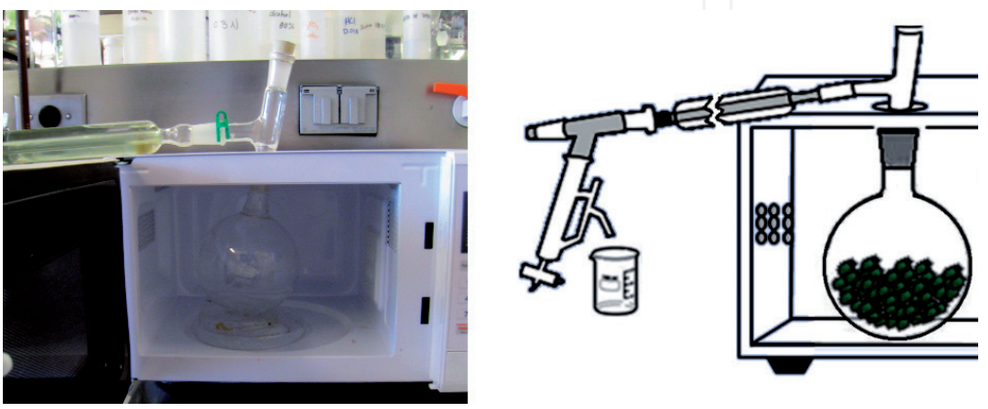

Figure 10.
Picture and schematic diagram of the microwave oven adaptation to perform MAE [60].

method increases the EO yield, ameliorate the EO composition, eliminate the waste of water treatment, and also contributes to limited time, and lower an energy consumption (**Figure 11**) [62].

2.3.1.2.7 Microwave hydrodiffusion and gravity

Microwave hydrodiffusion and gravity (MHG) is a new green extraction technique of EOs developed by Vian and collaborators in 2008. This green extraction technique is an original "upside down" microwave alembic combining microwave heating and earth gravity at atmospheric pressure [65]. MHG has become not only an economic and efficient but also an environmental- and eco-friendly, not require water or solvent and as it does require less energy (**Figure 12**) [65, 66].

2.3.2 Analysis of essential oils

As the consumption of EOs is growing up annually, their world production by different companies to satisfy the market demand has been increasing every year. The quality control of produced EOs has become then necessary to ensure the

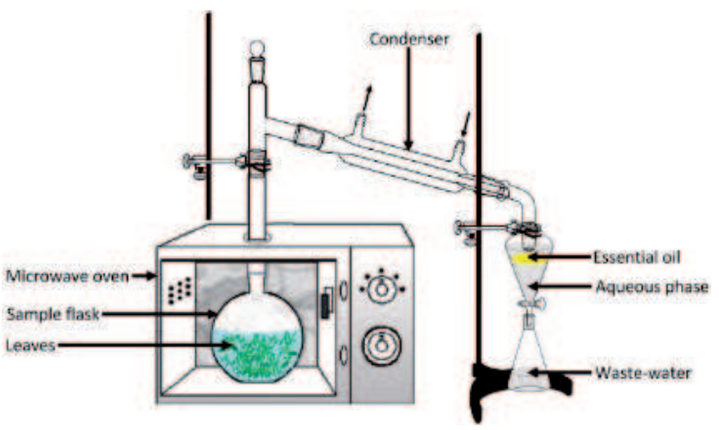

Figure 11.
Schematic representation of the solvent-free microwave extraction apparatus [63].

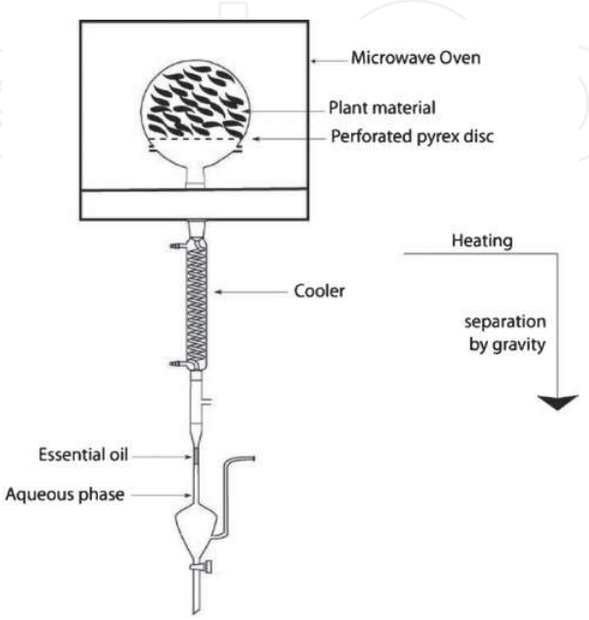

Figure 12.
Schematic representation of the microwave hydrodiffusion and gravity [65].

genuineness of the product, the shelf life, and the storage conditions [67]. The EO composition can sometimes be falsified by adding cheaper oils; it is often necessary to characterize small differences between oils that correspond to variation in geographic or genetic origin of the plant material. EOs analysis can be summarized in few points: the qualitative composition, the quantitative determination (major and/or minor constituents), and the detection of alteration of true EOs. With regard to the quality aspect of the EO, the identity and the purity are always investigated. Their physical properties are commonly assessed by specific gravity, the relative density, the optical rotation, the refractive index, etc.

Most of the methods applied in the analysis of EOs rely on chromatographic procedures, which enable component separation and identification. These include gas chromatography–mass spectrometry (GC–MS), liquid chromatography-mass spectrometry (LC–MS), gas chromatography-Fourier transform infrared spectrometry (GC-FT-IR), gas chromatography-Fourier transform infrared spectrometry-mass spectrometry (GC-FT-IR-MS), gas chromatography-atomic emission detector (GC-AED), gas chromatography-isotope ratio mass spectrometry (GC-IR-MS), on-line coupled liquid chromatography-gas chromatography (LC-GC), and multidimensional gas chromatography (MDGC) [68–78].

2.4 Bioactivities and toxicity of essential oils

A considerable large number of studies on EOs to evaluate their pharmacological properties and toxicity in order to find possible alternative medicine have become active in recent years [79]. EOs are known to exhibit a large range of biological activities.

2.4.1 Antioxidant activity

It is one of the most intensively studied properties of EOs. This could be explained by the damages of various biological substances by oxidation which subsequently causes many degenerative and/or metabolic diseases such as cancer, diabetes, arthritis, inflammation, and Parkinson's and Alzheimer's disease just to name a few [80–84]. EOs are known as rich sources of potential antioxidants that can be investigated to prevent oxidative damage [85]. Antioxidants comprise substances that, in low concentrations, significantly delay or inhibit the oxidation of the substrate [86]. Volatile compounds in EO, beside their protective antioxidant activity, can also act as prooxidant, by affecting the cellular redox status and damage cellular biomolecules, in the first instance proteins and DNA [15]. All these must be taken into account when antioxidant properties of EOs are considered.

Although phenolic compounds are recognized as being responsible for the antioxidant ability, recent studies showed that volatile components could also individually and/or in mixture (essential oil) contribute to the whole antioxidant ability. EO of lemon balm (*Melissa officinalis* L.) was reported to exhibit the highest antioxidant activity than BHT. Its GC–MS analysis showed that the main compounds were citronellal, neral, and geranial with a percentage yield of 13.7, 16.5, and 23.4%, respectively [87].

2.4.2 Anticancer activity

Cancer is a worldwide public health concern with 18.1 million people been diagnosed with the disease annually. It is the second largest single leading cause of death claiming in excess of 9.6 million lives in the world in 2018, with approximately 70% of deaths occurring in low- and middle-income countries [88]. Current

valuable drugs used in the treatment include vinblastine, vincristine, camptothecin, and Taxol [89]. Many studies pointed out the anticancer properties of plants. Over 500 research papers are published on the anticancer activity of EOs [90–93], even though, till date, there are no scientific studies showing that aromatherapy can cure or prevent cancer. Most promising research results obtained from in vitro studies revealed that EOs were found to affect cancer cell lines in petri dishes. EOs are well known for their anti-inflammatory activity; hence it appeared that EOs could also have anticancer effects as there is a relationship between the production of reactive oxygen species to the origin of oxidation and inflammation that can lead to cancer. More than 100 EOs from more than 20 families of plants have been tested on more than 20 different types of cancers in the past 10 years [94]. Bourgou and collaborators showed that the EO from seeds of black cumin (*Nigella sativa* L.) significantly inhibits the growth of A-549 and DLD-1 cancer cell lines with IC_{50} values of 43.0 and 46.0 μg/mL, respectively [95]. In 2012, Wang and collaborators reported the toxicology potential of EO of *Rosmarinus officinalis* L. and its three main components (including α-pinene, β-pinene, and 1,8-ceneole) toward three human cancer cell lines: the EO showed a strong cytotoxicity toward the three cancer cells with IC_{50} values of 0.025, 0.076, and 0.13‰ (v/v) on SK-OV-3, HO-8910, and Bel-7402, respectively [96].

2.4.3 Antimicrobial activity

EOs are well-kwon as antimicrobial agents and are well documented in numerous research works. Their antimicrobial activity depends not only on the presence of the main active compounds but also on the interaction between different components which can have synergistic or antagonistic actions. It also depends on the content, concentration, interaction between main active components, and susceptibility of microorganisms [97, 98]. The inactive compounds might influence resorption, the rate of the reactions, as well as biological activities of active compounds. The combination of both major and minor components can thus modify the activity to exert significant synergistic or antagonistic effect [99, 100]. EOs extracted from cinnamon, oregano, and thyme showed significant antibacterial activities against *Escherichia coli*, *Bacillus thermosphacta*, *Listeria monocytogenes*, and *Pseudomonas fluorescens* [101].

In general, EOs in decreasing order of antimicrobial activities are reportedly as follows: oregano (*Origanum vulgare*) > clove (*Syzygium aromaticum*) > coriander (*Coriandrum sativum*) > cinnamon (*Cinnamomum cassia*) > thyme (*Thymus vulgaris*) > mint (*Mentha*) > rosemary (*Salvia rosmarinus*) > mustard (*Sinapis alba*) > sage (*Salvia officinalis*) [102].

2.4.3.1 Antibacterial and antifungal activities

Antibiotic resistance is one of the most serious health burdens worldwide due to the continuous appearance of antibiotic-resistant bacterial strains. The bacteria that cause the most major clinical problems are *Klebsiella* and *Enterobacter* species, *Staphylococcus aureus*, *Enterococcus faecium*, *Clostridium difficile*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli* [103]. Generally, EOs are more active on gram-positive bacteria due to the presence of peptidoglycan layer, which lies outside the outer membrane. In gram-negative bacteria, the outer membrane is composed of a double layer of phospholipids, which is linked to the inner membrane by lipopolysaccharide [104]. Several studies on the bioactivity of EOs have revealed their antibacterial and antifungal potential on different pathogen microorganisms [105–108]. Previous studies revealed that the EOs from *Piper guineense*

fruit and *P. caldense* roots were active against the gram-negative bacteria *E. coli* and *P. aeruginosa* [109, 110]. EOs have been reported to possess potent antimicrobial activity, exhibiting bacteriostatic and bactericidal effects against tested pathogens.

2.4.3.2 Antiviral activity

New agents that are effective against common pathogens are needed particularly for those resistant to conventional antiviral agents. The ability of viruses to persist in fresh products, as well as their low infectious dose, could lead to serious food-borne problems [111]. Plants and plant-derived natural products provide unlimited opportunities for new antiviral drugs. Many EOs have been investigated in recent years toward their antiviral activity. As conclusion of their work, Reichling and collaborators reported that particular free viruses are very sensitive to EOs [112].

2.4.4 Anti-inflammatory activity

Most of EOs have been firstly identified and used for the treatment of inflammatory and oxidative diseases. *Cymbopogon citratus* (Lemongrass) is a popular herb used as analgesic and anti-inflammatory agent. It has been reported that its EO suppresses COX-2 expression promoter activity; citral was identified as the major component responsible for suppressing COX-2 expression and for activating PPARα and γ [113].

2.4.5 Miscellaneous activities

The insect repellent activity of EOs is well studied and many research papers have been published. The EOs of *Hyptis spicigera* Lamarck and *Hyptis suaveolens* (L) Poitier and *Lavandula angustifolia* (Miller) showed repellent activity on *Sitophilus zeamais* adults [114].

EOs of the leaves of *Endlicheria bracteolate* was tested against *Leishmania amazonensis* by Rottini and collaborators. The antileishmanial activity was evaluated against promastigotes and intracellular amastigotes, and cytotoxicity was performed with J774.G8, which were incubated with different concentrations of *E. bracteolate*. Promastigote forms showed *E. bracteolate* EO IC_{50} value of 7.945 μg/mL (24 h). The IC_{50} value was 15.14 μg/mL showing that *E. bracteolate* EO is less toxic to macrophages than to parasites [115].

2.5 Composition of essentials oils

EOs are generally very complex mixture (60–300) of nonpolar and semipolar lipophilic constituents of low molecular weight, at different concentrations with two or three appearing to be major ones [116, 117]:

- Terpenoids
- Straight-chain compounds not containing any side chain
- Aromatic and phenolic components
- Sulfured derivatives

The variation in odor and taste of EO depends on the plants variety, the harvesting seasons, the geographical location, the drying methods, and the extraction

techniques [102, 118–120]. The major volatile constituents may be classified into two main categories: terpenoids and polypropanoids [121–123]. We will focus our investigation on terpenoids.

3. Terpenes and terpenoids

Terpenes are defined as secondary metabolites with molecular structures containing carbon backbones of isoprene (2-methylbuta-1,3-diene) units [124]. Terpenes are synthetized in the cytoplasm of plant cells through the mevalonic acid pathway. Biochemical modification such as oxidation or rearrangement of terpenes produces the related terpenoids. Terpenoids are then oxygenated derivatives of hydrocarbon terpenes such as aldehydes, ketones, alcohols, acids, ethers, and esters [34]. Terpenoids are the largest classes of plants' natural products accounting for more than 40,000 individual compounds of both primary and secondary metabolisms been identified; to date, new terpenoids are being discovered every year [12, 124].

In general, terpenoids can be divided into at least four groups of compounds that include true terpenes, steroids, saponins, and cardiac glycosides.

These types of natural lipids can be found in every class of living things, mainly in plants as constituents of EOs, and are therefore considered as the largest and structurally diverse group of natural products [125]. In general, only the hemiterpenoids, the monoterpenoids, and sesquiterpenoids are sufficiently volatile to be components of EOs. As widely acknowledged, the composition of EOs is mainly represented by mono-, sesqui-, and even diterpene hydrocarbons and their respective oxygenated derivatives [30, 126–128].

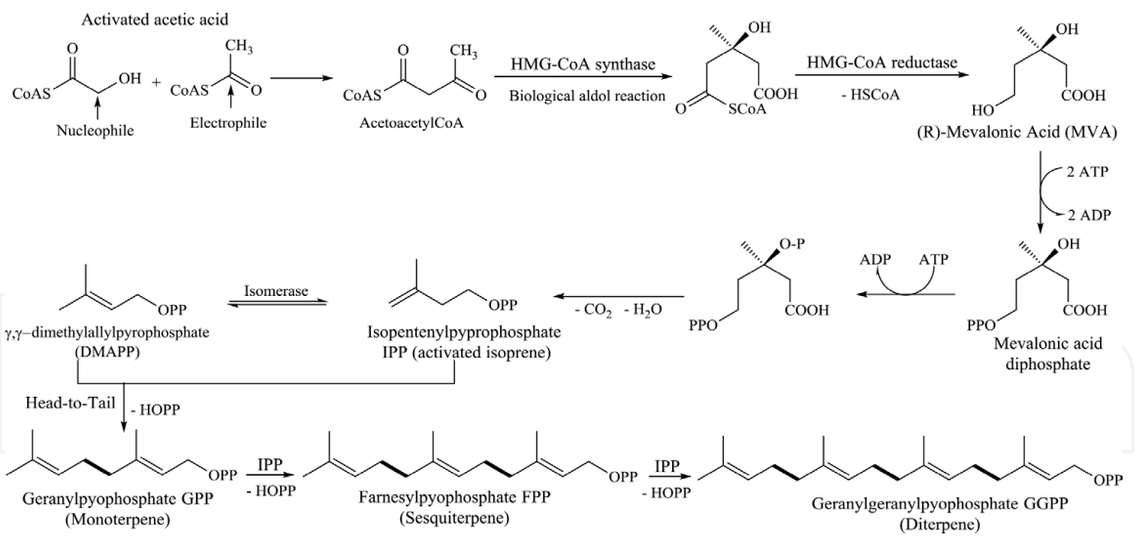

Figure 13.
Biosynthesis pathways of monoterpenes, sesquiterpenes, and diterpenes.

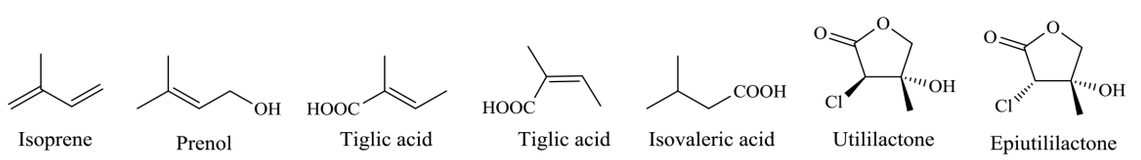

Figure 14.
Structure of few isolated hemiterpenes and hemiterpenoids.

3.1 Classification

Structurally, EO constituents typically have low molecular weights, which contribute to their high volatility. Terpenes are the most common constituents found in EOs [128]. They are made from isoprene units (several five carbon base units). Each group of terpenes arises from the head-to-tail condensation of a variable number of isoprene units. Variations in the number of isoprene unit repetitions, cyclisation reactions, and rearrangements are primarily responsible for their chemical and structural diversity. EOs consist of mainly monoterpenes (C_{10}) and sesquiterpenes (C_{15}) but also have diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}) at very low concentration with their oxygenated derivatives, respectively (**Figure 13**) [15, 102, 130].

3.1.1 Hemiterpenes

Hemiterpenes are part of minor terpenes of EOs. They are usually alcohols, aldehydes, and esters, with a 2-methylbutane skeleton [131]. The number of

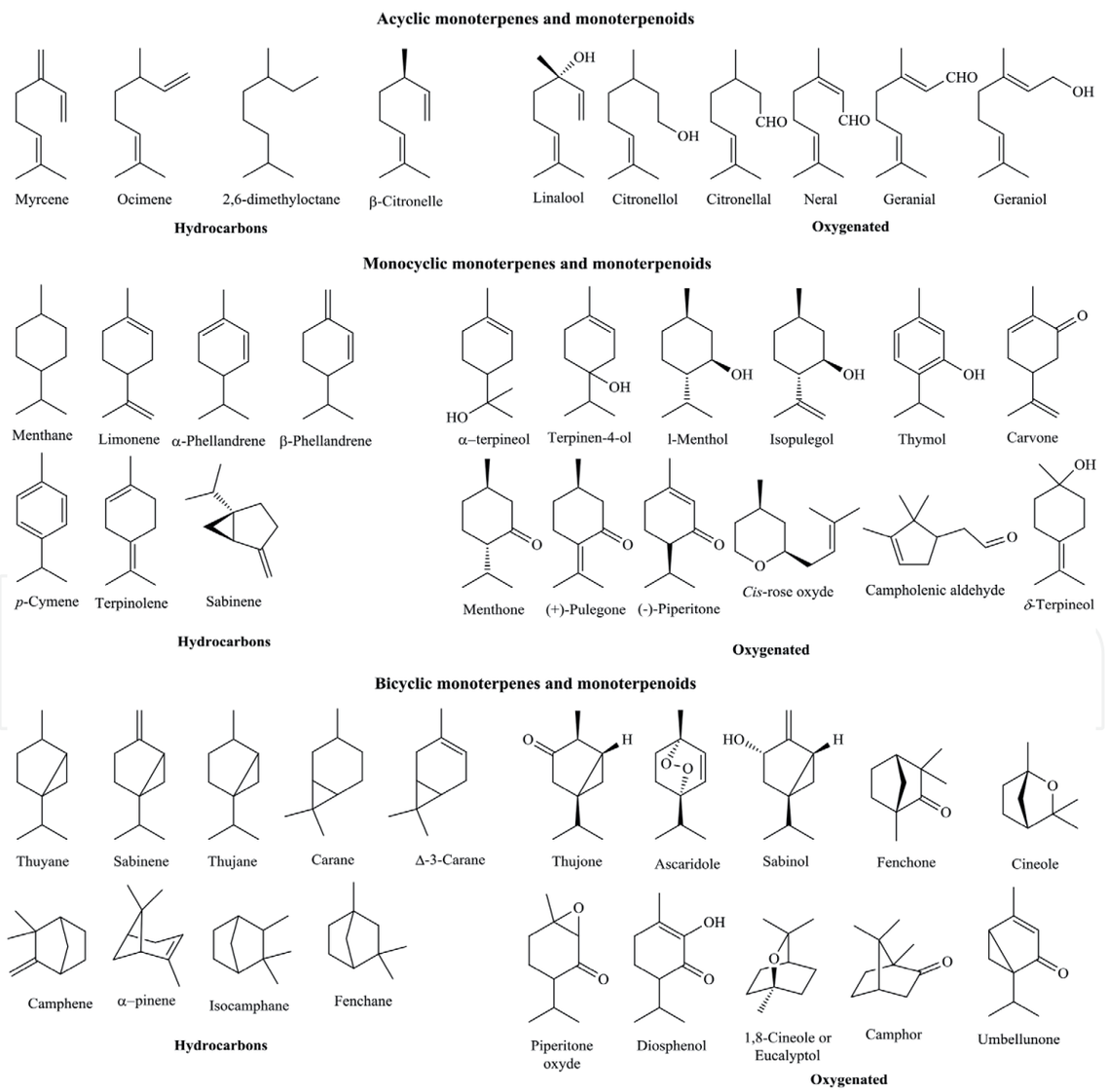

Figure 15.
Structures of some monoterpenes and monoterpenoids.

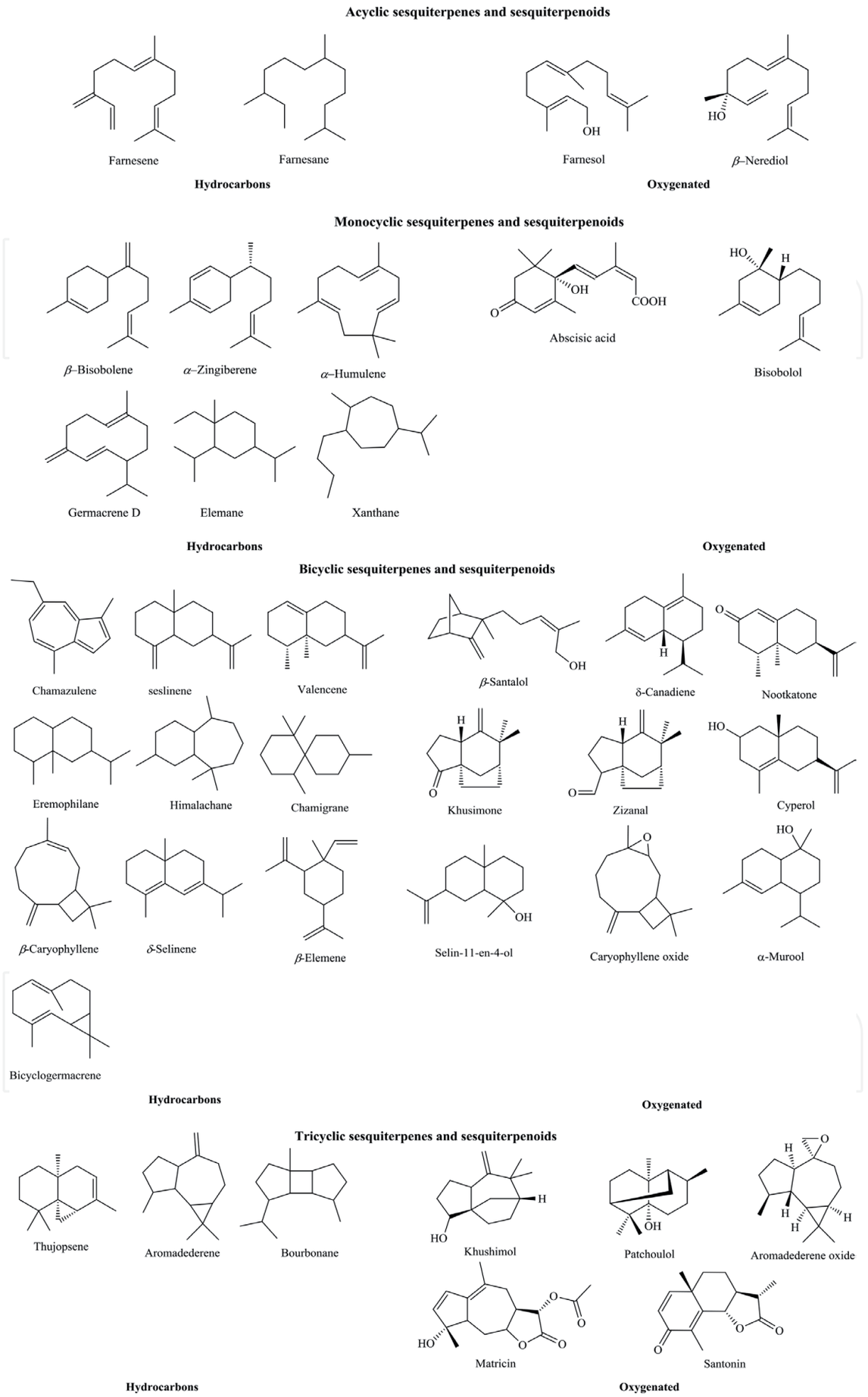

Figure 16.
Structures of some sesquiterpenes and sesquiterpenoids.

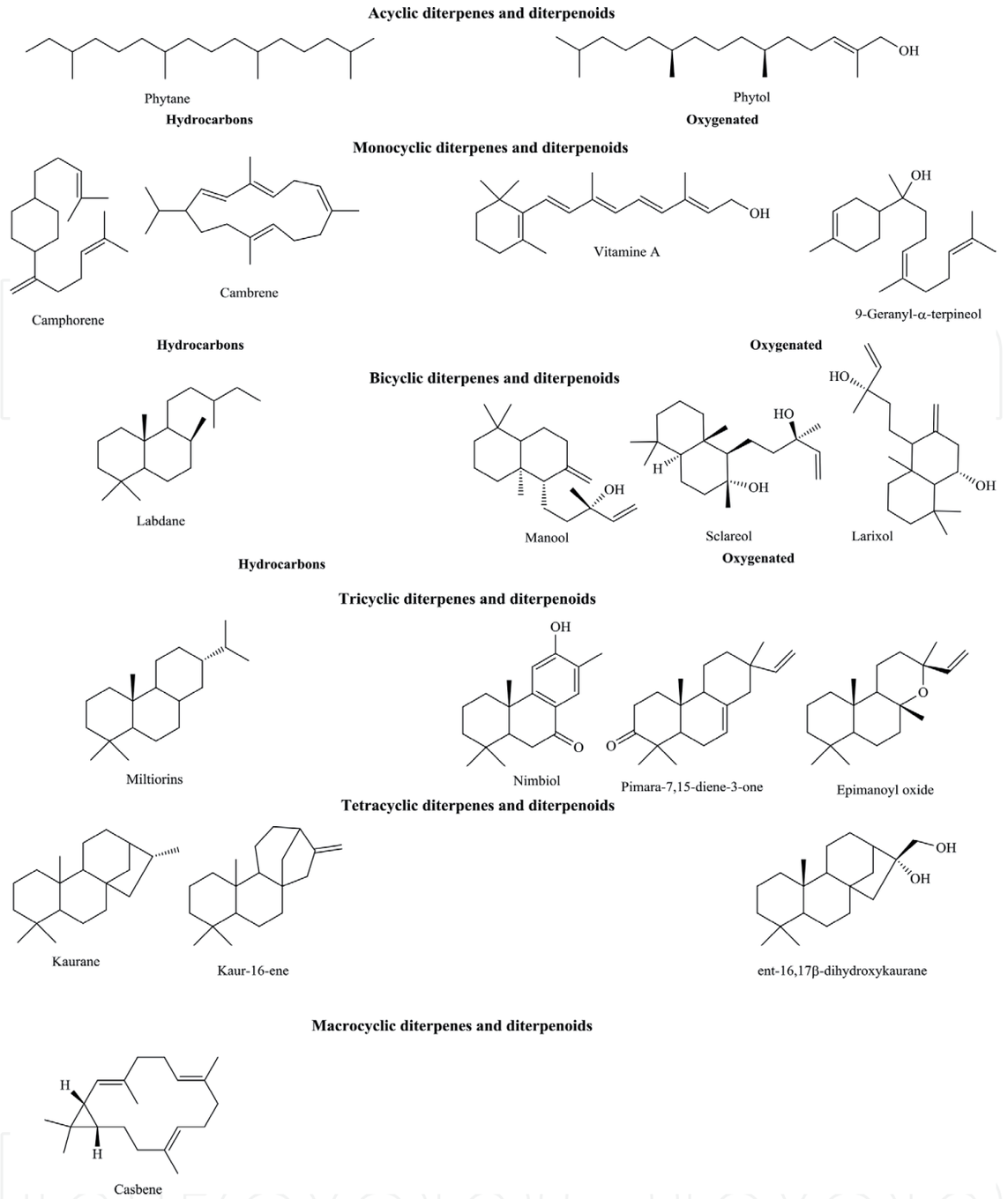

Figure 17.
Structures of some diterpenes and diterpenoids.

hemiterpene aglycone is less than 100 [132]. Chlorinated hemiterpenes were recently isolated from the leaves of *Prinsepia utilis* (**Figure 14**) [133].

3.1.2 Monoterpenes

Regular monoterpenes are made from the combination of two isoprene units (C_{10}) linked by the head-to-tail binding. They are the major molecules consisting of 90% of (some) EOs; thereby, they contribute to the specific smell of plants [134, 135]. Monoterpenes are found in nearly all EOs and usually possess one double bond in their structures. In nature, they are mostly involved in plant–animal and plant–plant interactions such as pollination, seed and fruit dissemination, and allelopathic agents. Monoterpenes occur in more than 30 known

skeletons and can be divided into 3 subgroups: acyclic, monocyclic, and bicyclic. A number of monoterpenes are oxygenated (**Figure 15**).

3.1.3 Sesquiterpenes

Sesquiterpenes are other major EO components and are less volatile than monoterpenes. They are derived from three isoprene units and exist in a wide variety of forms, including linear, monocyclic, bicyclic, and tricyclic frameworks. Sesquiterpenes are the most diverse group of terpenoids (**Figure 16**).

3.1.4 Diterpenes

They are chemically complex and are usually components of plants resins but are sometimes encountered as by-products in the isolation of EOs. Diterpenes are less volatile because of their high molecular weights and less numerous than the mono- and sesquiterpenes. Consequently, they are difficult to extract by steam distillation and then appear rarely in distilled EOs. When present, they are found in EOs in very low amounts. However, traditional extraction using distillation allows separation and identification of diterpenes present in EOs [136]. Generally, molecules with molecular masses higher than 300 uma can be seen as sign of improper extraction conditions or adulteration. Diterpenes that are usually found in EOs include camphorene, cafestol, kahweol, cambrene, and taxideme (**Figure 17**).

3.2 Bioactivities of terpenoids

Some sesquiterpenoids are very toxic, but some are antifungals, carminatives, and insecticides.

Being complex mixtures of constituents, overall activities of EOs cannot therefore be attributed only to their major components (terpenoids) [137]. Many aroma components of EOs, such as terpenes and terpenoids, were proposed to contribute to their antioxidant activity; that include β-terpene and β-terpinolene in *Melaleuca alternifolia*, 1,8-cineole in *Mentha aquatic*, and linalool in black cumin. Less volatile but strongly bitter-tasting or toxic terpenes also protect some plant from being eaten by animals. Some terpenes are potent drugs against diseases such as heart disease, malaria, and cancer [34].

4. Importance of terpenes terpenoids found in essentials oils

Terpenoids are, by far, the most important group (numerous and structurally diverse) of natural products as far as EOs are concerned. Reports on the level of terpenoids in EOs vary considerably. Many terpenes have biological activities and are used for medical purposes. For example, the antimalarial drug artemisinin and the anticancer drug Taxol (paclitaxel) are two of a few terpenes with established medical applications [26].

Monoterpenes are well known as main constituents of EOs, floral, and scents. Monoterpenes and monoterpenoids have antioxidant, anticonvulsant, antiulcer, anti-inflammatory, antiseptic, antitumor, antiviral, analgesic, antihypertensive, antibacterial, and therapeutic antidiabetic properties [26, 138]. The general mechanism of action of monoterpenes, such as their antimicrobial and antitussive activity, is mainly related to their volatility. Their hydrophobicity, as well as the EOs as a whole, determines their effect on bacterial cell structures with a subsequent antimicrobial effect [139]. α-Terpineol is used to enhance skin penetration and

also has insecticidal properties [140]. Monoterpenes have been shown to exert chemopreventive as well as chemotherapeutic activities in mammary tumor models and thus may represent a new class of therapeutic agents [138]. The EO of *Melissa officinalis* L. can inhibit the replication of HSV-2, due to the presence of citral and citronellal [141]. Linalool is an unsaturated alcohol monoterpene found as principal constituent in many EOs known to exhibit various biological activities that include antibacterial, antiplasmodial, and antinociceptive effects in different animal models [142–144]. Linalool also plays an important role in nature as a key compound in the complex pollination biology of various plant species to ensure reproduction and survival. It is also a key compound for the industrial production of a variety of fragrance chemicals such as geraniol, nerol, citral and its derivatives, as well as a lead compound in the synthesis of vitamins A and E. Its repellent properties on various crop-destroying insects are well studied and documented, hence accentuating the application of linalool in eco-friendly pest management [145]. In Malaysia, linalool is reported to be the major component of EOs of different aromatic species of the Lauraceae family; hence it may be classified as a taxon of this family [146]. Limonene is among the most abundant monoterpene constituents found in nature, and it occurs in a variety of trees and herbs that include *Citrus* species. It has been an interesting target molecule for chemists and biologists. Limonene inhibits LPS-induced NO and PGE2 production that included dose-dependent decreases in the expression of iNOS and COX-2 proteins [147]. Some in vitro and in vivo studies have revealed the effects of monoterpenes on diabetes, insulin resistance, and obesity. The role of inflammation as a link between diabetes and obesity has been established. Many monoterpenes exhibit ameliorative effects in inflammatory conditions associated with diabetes [148]. The analgesic effect of many plant EOs rich in monoterpenes has been established experimentally [149].

Some bicyclic monoterpenoids are known to suppress the acetylcholinesterase activity, which is increased in patient with Alzheimer's disease. In a study of 17 monoterpenes and monoterpenoids, (+)- and (−)-α-pinene and (+)-3-carene appeared as potent inhibitors of the enzyme AChE, while the bicyclic ketones and alcohol inhibitions were weak [150].

In recent years, a considerable large number of research studies have been carried out on the chemical constituents of EOs as source of bioactive natural products against cancer. Piaru and collaborators showed that EO of *Myristica fragrans* exhibited good cytotoxic activity, possibly due to the presence of some potential anticancer substances such as limonene, terpinen-4-ol, eugenol, and myristicin [151]. Similarly, EO from *Vepris macrophylla* demonstrated a strong cytotoxic effect, which may be attributed to the presence of specific components like citral, citronellol, and myrcene [152, 153].

Many EO components possess enantiomers that can be sometime present in an oil. It is important to note that there is a close relationship between the chirality of organic compounds and their biological properties. For a given optically active substance, the activity is not identical for both enantiomers [153]. Linalool, for example, has two enantiomers: (3*S*)-(+)-linalool known as coryandrol and (3*R*)-(−)-linalool known as licareol. Both have distinct properties. It was reported that although (*S*)-(+)- and (*R*)-(−)- have similar activity profiles, the effect of (*R*)-(−)-linalool is more intense [154]. Similarly, De Sousa and coworkers showed that regarding the anticonvulsant activity, (*R*)-(−)-linalool and the racemate form were more active than the (*S*)-(+)- enantiomer, which had effects compatible with diazepam and phenytoin, known as anticonvulsant agents [148, 153].

Geraniol, an acyclic aldehyde monoterpene present in various EOs from many aromatic plants, has in vitro and in vivo antitumor activity against several cancer cell lines. In fact, geraniol alters several metabolic pathways of HepG2 cells such

as the mevalonate pathway and the phosphatidylcholine biosynthesis, which results in cell growth inhibition, cell cycle arrest occurring at the G0/G1 interphase, and increased apoptosis [155]. Antibacterial and antifungal activities of oils with high levels of sesquiterpenes as cadinene, spathulenol, and selinene were described [156].

Cristiani and coworkers have reported the antimicrobial activity of four monoterpenes (*p*-cymene, *γ*-terpinene, carvacrol, and thymol) against the Gram-positive bacterium *S. aureus* and the Gram-negative bacterium *E. coli*. They concluded that thymol was considerably more toxic against *S. aureus* than the other three terpenes, while carvacrol and *p*-cymene were the most active against *E. coli* [157]. Germacrene D with its three double bonds as electron-rich centers demonstrated good ability to scavenge superoxide radical anions [156]. However, linalool and nerolidol may also display pro-oxidant activity. Carvacrol and thymol are reported to be the main constituents of volatile oils from *Origanum* species in general [158, 159]. With limonene, citronellol, myrtenol, linalool, and carvacrol are among monoterpenes showing in vitro and in vivo cardiovascular effects in both humans and animals [159].

Monoterpenes, sesquiterpenes, and oxygenated derivatives extracted from EOs have shown strong inhibitory activities against pathogenic bacteria, hence suggesting their use as flavoring and antioxidant agents [104].

Alzheimer's disease is by far the most prevalent of all known forms of dementia. Wojtunik-Kulesza and collaborators showed that three monocyclic monoterpenes (carvone, pulegone, and γ-terpene) possess acetylcholinesterase (AchE) inhibitory activity. Among the investigated terpenes, the three later were recognized as compounds with promising activities in the development of multi-target directed ligands [160]. The lipophilic character of terpene skeleton combined with the hydrophobic character of the functional group is essential for activity. Thus, a rank of activity has been proposed as follows: aldehydes > ketones > alcohols > esters > hydrocarbons [156].

In 2010, Conti and coworkers measured the insect repellent activity of three EOs. They found that at lowest dose (0.001%), the OE of *Hyptis suaveolens* exhibited a significant higher repellent effect compare to *Hyptis spicigera* and *Lavandula angustifolia*. After chemical analyses of the OE of *H. suaveolens*, monoterpene hydrocarbons were the most represented class of volatiles (64.1%), followed by sesquiterpene hydrocarbons (24.0%) [114].

In EOs, the components found in higher concentrations and related to antimicrobial activity are phenolic compounds such as linalool, sabinene, menthol, myrcene, and camphene [161].

Sesquiterpenes have anti-inflammatory and anti-allergic properties. The anti-inflammatory activities of some medicinal plants are due to the presence of one or more sesquiterpene lactones [26]. Above all, terpenes are responsible for the smell and flavor typical of the different varieties of *Cannabis sativa*, whereas phytocannabinoids are odorless [162].

5. Conclusion

Terpenes represent one of the largest and most diverse classes of natural products. They have numerous roles ranging from defense repellents against herbivores or pathogens through animal attract hormones to agents designed to help disperse seeds and pollen. Monoterpenoids and sesquiterpenoids are obviously the major constituents of EOs, while in some oils the occurrence of diterpenoids was observed as quite minor constituents when present. In an ecological context, mono- and

sesquiterpenes play an important role in the relations between organisms, for example, as attractants of pollinators or deterrents of herbivores. The enormous diversity of terpenoids and wide spectrum of biological activities make them attractive for many industries, and new areas of application still have not been discovered. Despite their rich and complex composition, the use of EOs remains limited to the cosmetics and perfumery domains. It is worthy to develop a better understanding of their chemistry and biological properties as well as that of their individual components for new and valuable applications in human health.

Despite their well-recognized bioactivities, EOs have been misused with regard to their level of toxicity. Some EOs or their major constituents have been recorded to be much toxic with bad side effects including convulsions, irritation, and photodermatosis. Literature review of the available data shows that serious accidents, most of which involve young children, are due to a small number of EOs, ingested in large amount. The development and the expansion of therapies using EOs and the evaluation of their acute toxicity have become more important to avoid their abusive use. The most common adverse events are eye, mucous membrane, and skin irritation and sensitization particularly to oils containing aldehydes and phenols. Despite all, no well-defined studies have proved that these EOs are harmful, but this deserves more detailed studies.

Author details

Fongang Fotsing Yannick Stephane[1*] and Bankeu Kezetas Jean Jules[2]

1 Department of Chemistry, Higher Teachers Training College, University of Maroua, Maroua, Cameroon

2 Department of Chemistry, Faculty of Science, University of Bamenda, Bambili, Cameroon

*Address all correspondence to: fongangfys@yahoo.fr

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